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NOVEL beta-ACTIN AND RPS21 PROMOTERS AND USES THEREOF

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Inventor(s):		
Applicant(s):		
Classification:	**- international:**	***A01K67/027; A61K48/00; C07H21/04; C07K14/47; C07K16/22; C12N15/09; C12N15/79; C12N15/85; C12N5/06; C12N5/10; C12N9/02; C12N9/16; C12N9/24; C12P21/02; C12P21/08***
	- European:	A61K48/00D; C07K16/22
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Priority number (s):	US20030480768P 20030624; WO2004US17422 20040624	
Also published as:	• WO 2005000888 (A2) • WO 2005000888 (A3) • US 2005026252 (A1) • US 7423135 (B2) • US 2008301826 (A1) • more	

Abstract not available for JP 2007525956 (T)
Abstract of corresponding document: WO 2005000888 (A2)

The invention relates to isolation of novel beta-actin and ribosomal protein S21 (rpS21) promoters and uses thereof. In particular, this invention features nucleotide sequences for rodent beta-actin promoters including, hamster, rat, and mouse, and hamster rpS21 promoter.

Last updated: 26.04.2011 Worldwide Database 5.7.22; 92p

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C12N 9/16	**(2006.01)**	C12N	9/16	D	4B064
C12N 9/24	**(2006.01)**	C12N	9/24		4B065
C12P 21/02	**(2006.01)**	C12P	21/02	C	

審査請求 有　予備審査請求 未請求　（全 39 頁）　最終頁に続く

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(33)優先権主張国　米国(US)

(71)出願人　505187264
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弁理士　清水　初志
(74)代理人　100128048
弁理士　新見　浩一
(72)発明者　エスティス　スコット　ディー．
アメリカ合衆国　マサチューセッツ州　フレーミングハム　ウィンチ　ストリート　３７

最終頁に続く

(54)【発明の名称】新規β－アクチンおよびＲＰＳ２１プロモーター、ならびにこれらの使用方法

(57)• • • • • • • •
• S21• rpS21• •
• •
• rpS21• •
• •

1 2 3

1

2

3

1

2

1

2

3

7 9

10

11

7 12

CHO 13

a

b

15

T6F 16

15

18

16

20

10

39 rpS21

39

23

24

20

25

23 26

CHO 27

a rpS21

30

b

29

29

31

40

22

33

ATCC PTA-5309

ATCC rpS21

50

S21

2003 6 24 60/480,768

10

CMV RSV 40 SV40 20

Fernandez et al. 1999 Gene Expression System
s, Academic Press

CMV

Xu et al. 2001 Gene 272 149-156 30

CMV SV40

CMV
Xu et al.,

S21 rpS21 40S

rpS21 GenBank

AJ250907 TATA

CAAT Smirnova et al. 2000
Bioorg. Khim. 26 5 392-396

40

rpS21

rpS21

CHO

rpS21

CMV 50

rpS21

rpS21

rpS21

10

ASM GAA

tPA

CHO HEK BHK

20

rpS21

ASM GAA tPA

30

5'

3'

5' 3'

40

PCR

mRNA

ELISA Bradford

Bradford 1976 Anal. Biochem., 72 248

50

Molecular Cloning: A Laboratory Manual, 2nd edition, Sambrook et al., 1989
Cold Spring Harbor Laboratory Press.

45% 50% 55% 60% 65
%, 70%, 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%

5%, 70%, 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%

3

3, nt 1, nt 2953, 55%, 60%, 65%, 70%,
75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%

39, rpS21, 39, nt 1, nt 1958,
40%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 9
8%, 99%

10

1

1250, 5', 20,
1, nt 50, nt 3000, nt 100, nt 3000, nt 150, nt 3000, nt 200, nt 3000,
nt 250, nt 3000, nt 500, nt 3000, nt 1000, nt 3000, nt 1500, nt 3000,

2

5', 2, nt 50,
nt 2490, nt 100, nt 2490, nt 150, nt 2490, nt 200, nt 2490, nt 250, nt 2490, nt
500, nt 2490, nt 1000, nt 2490,

3, 5',
3, nt 50, nt 2950, nt 100, nt 2950, nt 150, nt 2950, nt 200, nt 2950, nt
250, nt 2950, nt 500, nt 2950, nt 1000, nt 2950, nt 1500, nt 2950, 30,

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1, 2, 3,
1250, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900,
1950, 2000, 2500, 3000,

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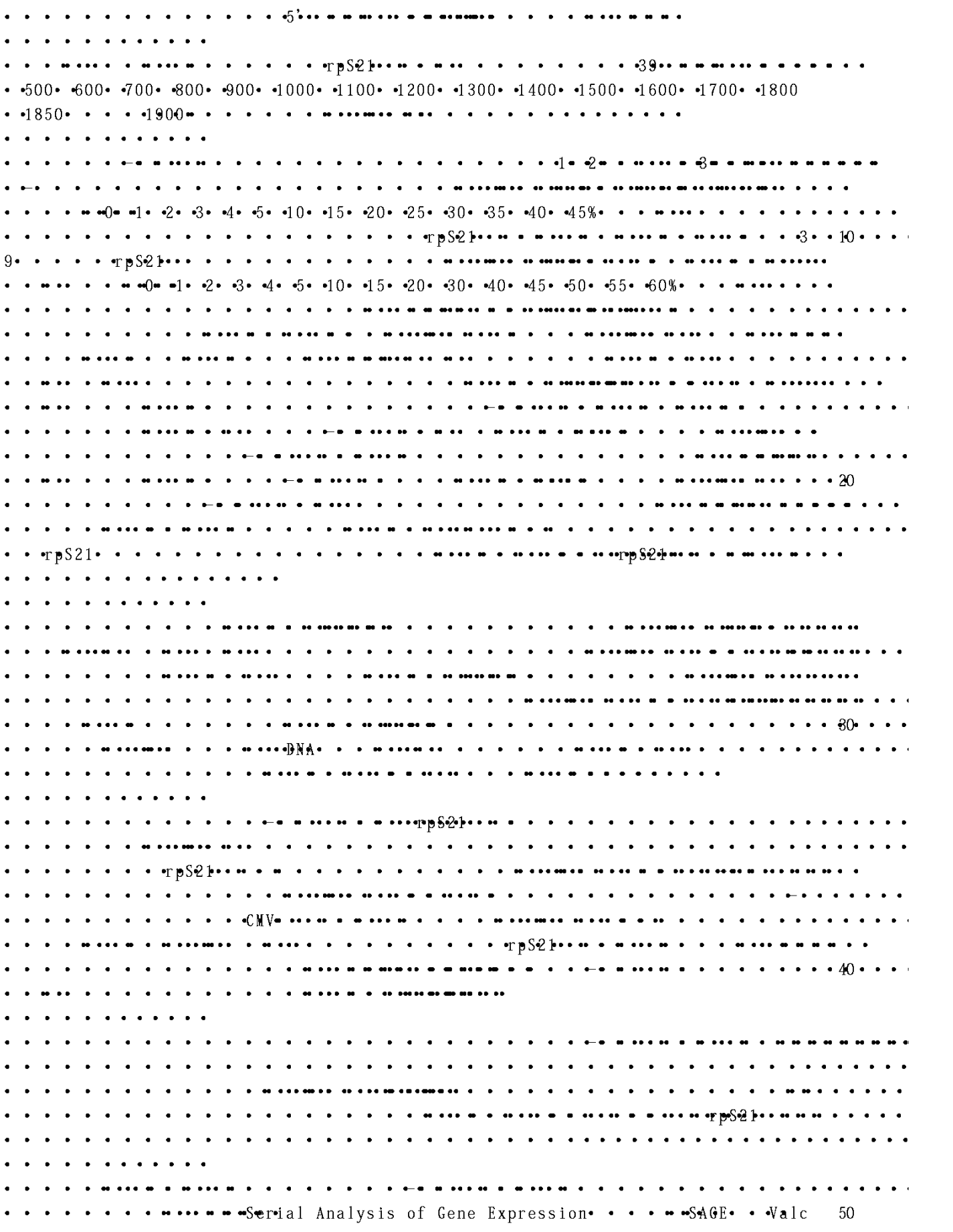

5'
rpS21
3'
500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800
1850 1900
1
2
3
0 1 2 3 4 5 10 15 20 25 30 35 40 45%
rpS21
3
10
rpS21
0 1 2 3 4 5 10 15 20 30 40 45 50 55 60%
20
rpS21
rpS21
30
DNA
rpS21
rpS21
CMV
rpS21
40
rpS21
Serial Analysis of Gene Expression
SAGE
Valc
50

ulesco et al. (1995) Science, 270• •484-487• • • •Valculesco et al. (1987) Cell, 88: 243-251• • • • •CHO• • • •

• • • •SAGE• • • • •SAGE• • • •CHO• • • • •CHO• • • • •CHO• • • • •rpS21• • • •

• • • •rpS21• • • •

• • • •

• • •1• •2• •3• • • •39• • • •1• • • •Tm• • • •

• •1%• • • •1• •1.5• • • •Bonner•et•al.• •1973• • J. Mol. Biol., 81• •123• • • •

• • • •1• •2• •3• • • •39• • • •

• • • •1• •2• •3• • • •39• • • •

• • • •1• •2• •3• • • •39• • • •

• • • •

• • • •G• •C• • • •DNA• • • • • • • •Howley•et al. (1979) J. Biol. Chem., 254• •4876• • •50%• •DNA• • • •

$$T_m = 81.5 + 16.6 \log M + 41(\%G + \%C) - 500/L - 0.62F$$

• •

• • • •

• •M• • • •

• • •%G + %C• • • •G• • •C• • • •

• •L• • • •DNA• • • •

• •F• • • •

• • • •

• •Ausubel et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, sections 2, 4, • • • •6• • • •

• • • •

• •Sambrook et al. (1989) Molecular Cloning• • A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, chapters 7, 9, • • • •11• • • •

• • • •

• • • •

• • • •DNA• • • •35%• • • •5• •SSC• •50mM• • • •HCl• •pH 7.5• • •5• mM EDTA• •0.1% PVP• •0.1% Ficoll• • • •1% BSA• • • •500• •g/ml • • • • DNA• • • •40• • • •6• • • •

0.02% PVP 0.02% Ficoll 0.2% BSA 100 g/ml
DNA 10% wt/vol 5 20 10^6 ^{32}P- 40 18 20
2 SSC 25mM -HCl pH 7.4 5mM EDTA 0
.1% SDS 55 1.5
60 1.5
10
DNA 6 SSC 50mM -HCl pH 7.5 1mM EDTA 0.02% PVP
0.02% Ficoll 0.02% BSA 500 g/ml DNA
65 8 100 g/ml DNA
5 20 10^6 cpm ^{32}P
65 48 2 SSC 0.01% PV
P 0.01% Ficoll 0.01% BSA 37 1
0.1 SSC 50 45
20
5% SDS 1.0mM EDTA pH 8.0 42 50% 6 SSC
60 0.5 SSC 0.1% SDS
1 2 3 39
2
30
Devereux et al. 1984 Nucl. Acids. Res., 12:387
University of Wisconsin Genetics Computer Group UWGCG
GAP Tatusova et al. (1999) FEMS Microbiol. Lett.
, 174 247 BLAST www.ncbi.nlm.nih.gov/
BLAST 2
BLAST reward 2
penalty -2 ope
n gap extension gap 5
2 x dropoff 50 expec
40
t 10 word size 11
2 3
1 -- nt 1 nt 300
7 67% 80%
50

BLAST GenBank U 10
20114 1232
98%
40%
BLAST rpS21 GenBank AJ250907 nt 1 2344
39 rpS21 nt 1 1958
39 rpS21 rpS21 rpS21 Ge 20
nBank NT_039212 DNA
2 1
39 nt 1775 nt 1945 172nt 137 2
39 nt 580 nt 851 274nt 208 2
39 928nt N
T_039212 1745nt
39 nt 1775 nt 1945
39 nt 580 nt 851 80
nt 852 nt 1774 39
1 2 3 39
1 2 3 39 1
40
T6F 1 2 3
T6F

DNA

DNA

DNA

10

bic

stronic

2

1

1

2

2

2

IRES

mRNA

2

20

2

IRES

pS21

30

Discosoma striata

RFP

40

HeLa

CHO

NSO

HEK

BHK

NIH-3T3

MDCK

COS

50

DEAE-

1

10

DHFR DHFR DHFR

MTX DHFR

DHFR

DHFR

2

GS 20

GS

MSX

Ausubel 1995,

30

40

A. CHO-K1

CHO-K1 American Type Culture Collection Manassas, VA ATCC CRL-96
18 10% DGS Invitrogen 925
15g/L DE-52 Whatman, Kent, UK 250ml
20 40% O_2 5% CO_2 37
60rpm 6 80
% v/v 925 RNA 50

1 mRNA 7 mg/L D

B. RNA

RNA Promega Madison, WI RNAgents CHO-K1

5 g RNA NorthernMax Gly Ambion, Austin, TX

glycoxal

RNA Schleicher & Schuell, Dassel, Germany

PCR 10

GenBank M96676 nt 14-383

Genbank U20114 nt 238-381 EF-1 GenBank

D00522 nt 7-192 rpS21 GenBank

X79059 nt 68-340 GenBank

M99692 nt 182-303 3-

GAPDH Ambion Austin TX PCR

PCR

1

20

遺伝子	プライマー	配列	配列番号：
β-アクチン	フォワード	GCTCTTTCTTCGCCGCTCC	8
β-アクチン	リバース	ACCACCCTCCAGCCTTCCC	9
EF-1	フォワード	GAACGCAGGTGTTGTGAAAA	10
EF-1	リバース	CTCGGCAGCCTCCTTCT	11
rpS21	フォワード	GTGGACCTGTACGTGC	12
rpS21	リバース	TTCTCACTTTTATTTATGAC	13
フェリチン	フォワード	CGCCAGAACTACCACCAGGAC	14
フェリチン	リバース	TTCAGAGCCACATCATCCCG	15
ガレクチン	フォワード	TGGTCGCAAGCAACCTGAATC	16
ガレクチン	リバース	TTGAAGTCACCGTCTGCCGC	17

30

C. CHO-K1

CHO-K1 10% FBS Invi

trogen 925 6 Lipofectamine

Invitrogen 50-75%

pDsRED-1 Clontech, Palo Alto, CA

GD20 pSV40-CD

20 pDsRED-1 Discosoma stri 40

ata RFP FACS

Opti-MEM Invitrogen 16 -DNA

10% FBS 925 48

D.

FACS 1- 10^6 2% FBS PBS

30 FITC CD20 Pharmingen, San Diego,

CA 2% FBS PBS 1ml 50

PBS/2% FBS FACS FACSCalibur BD Biosciences, San Diego, CA CB20

E. ASM

ASM 12.5mM 2- N- 4- Calbiochem, San Diego, CA 0.1mM 0.25mg/ml BSA 0.1% Tween 20 250mM pH 5.5 37 50% 0.2M Na 10 OH ASM 145nm 2- N- 4- -

F. GAA

GAA 40mM p- -D-a Sigma, St. Louis, MO 0.1% BSA 50mM pH 4.3 37 0.3M pH 10.6 GAA 100nm 20 p

1. CHO-K1 SAGE CHO-K1 SAGE CHO-K1 mRNA DNA cDNA NlaIII cDNA 3' 30 cDNA I FokI II 20 II cDNA 10

3' NlaIII 10bp 40 SAGE cDNA SAGE M13 GTTTTCCCAGTCACGAC 18 PCR PCR pCR2.1 Invitrogen GenBank PCR www.ncbi.nlm.nih.gov/genbank

BLAST www.ncbi.nlm.nih.gov/blast 16 2- 15 5- 50

存在量	タグ	遺伝子	配列番号:	同定
38	CATGGAAGCAGAAT	Alu反復	19	J00052
33	CATGCAGGAGCTTC	Mito COX I	20	PCR
27	CATGGGGGAGCGTT	リボソームタンパク質S21	21	PCR
27	CATGGTACTGACAC	Mito COX III	22	PCR
20	CATGGCCTCCAAGG	GAPDH	23	X52123
20	CATGATAATACGTA	Mito ATPase 6	24	M14311
19	CATGCCTTTAATCC	B-1反復	25	PCR
18	CATGAATCGGAGGC	Mitoシトクロム B	26	J01436
18	CATGAGGCAGACAG	EF-1	27	D00522
18	CATGGCGGCAGACG	ガレクチン(L-14)	28	M96676
16	CATGGTGGCTCACA	Alu反復	29	J00056
15	CATGTTGGCTGCCG	フェリチン重鎖	30	M99692
14	CATGCCCTGTGCCG	マッチなし	31	
13	CATGAGAGCGAAGT	リボソームタンパク質L41	32	X82550
13	CATGAGGAGGCCTA	ミトコンドリアNADHデヒドロゲナーゼ	33	PCR
12	CATGCCCTGAGTCC	β-アクチン	34	AF014363

2 rpS21 rpS21 1 rpS21 mRNA rpS21 DNA FlpIn CHO-K1 Stratagene, LaJolla, CA rpS21 XL1-Blue MRA P2 10mM 0.2% LB 600nm 0.5 10mM 1,000,000 37 15 BioAssay Nunc, Rochester, NY 30 Genescreen Plus Perkin Elmer Life Sciences, Wellesley, MA 2 1.5M 0.1M 2 UV

GenBank U20114 nt 238-381 5' PCR rpS21 12 13 PCR rpS21 DNA 1 1

DNA 5'

7kb AvrII 5.5kb SalI pBluescript II KS Stratagene 7kb AvrII ATCC PTA-5309 2003 7 3 American Tissue Culture Collection, P.O.Box 1549, Manassas, VA 20108, U.S.A

AvrII SalI SfoI 3' pDsRED-1 Clontech pDsRED-Avr 6.5kb pDsRED-Avr 5.1kb 1 PCR

フォワード:AGGCCCAGCTTGGGACCAAGACAGAA (配列番号:35)

リバース:CGCGGATCCGGCGAACTATATCAGGGC (配列番号:36)

PCR 2 7kb 3kb PCR pDsRED-1 Clontech pDsRED-Avr 1 7 pDsRED-Avr 1 3

・フォワード:AGCTCTAATACGACTCACTATAGGGC (配列番号 :40)

・リバース:CTCTAGGCCAGCGGAGCGCAG (配列番号 :41)

細胞株	CMVプロモーター	β-アクチン・プロモーター
BHK-21	8.3 ± 0.4	121 ± 99.8
HEK293	139 ± 9.9	102 ± 8.3

ED-1 Clontech CMV pDsRED-1
RFP CHO-K1
RFP 48 FAC
S
CMV RFP
7
10
DHFR MTX
SV40 DHFR 3kb
1 pGZ6 pGLHAXSV2DHFR
pGLHAXSV2DHFR Cole et al. 1993 Biotechnology, 11 1014
1024 pGLHAXSV2DHFR
MT pGZ6
2 ASM
GAA cDNA
ASM cDNA IMAGE GenBank
AI587087 GAA cDNA New York University Schoo 20
l of Medicine Dr. Martinuik ASM GAA cDNA
37 38 2 cDNA DHFR
CMV DHFR CHO-K1
DXB11 3 20nM MTX
2 P
BS 24 ASM GAA
8A 8B
ASM GMV 30
2 15 GAA 2 5
MTX
1
20nM MTX ASM 10
MTX 200nM 2 4 3
2 20nM ASM 2 40
3 CMV 1
20nM 2
6 ASM
CMV
ASM 6
CMV

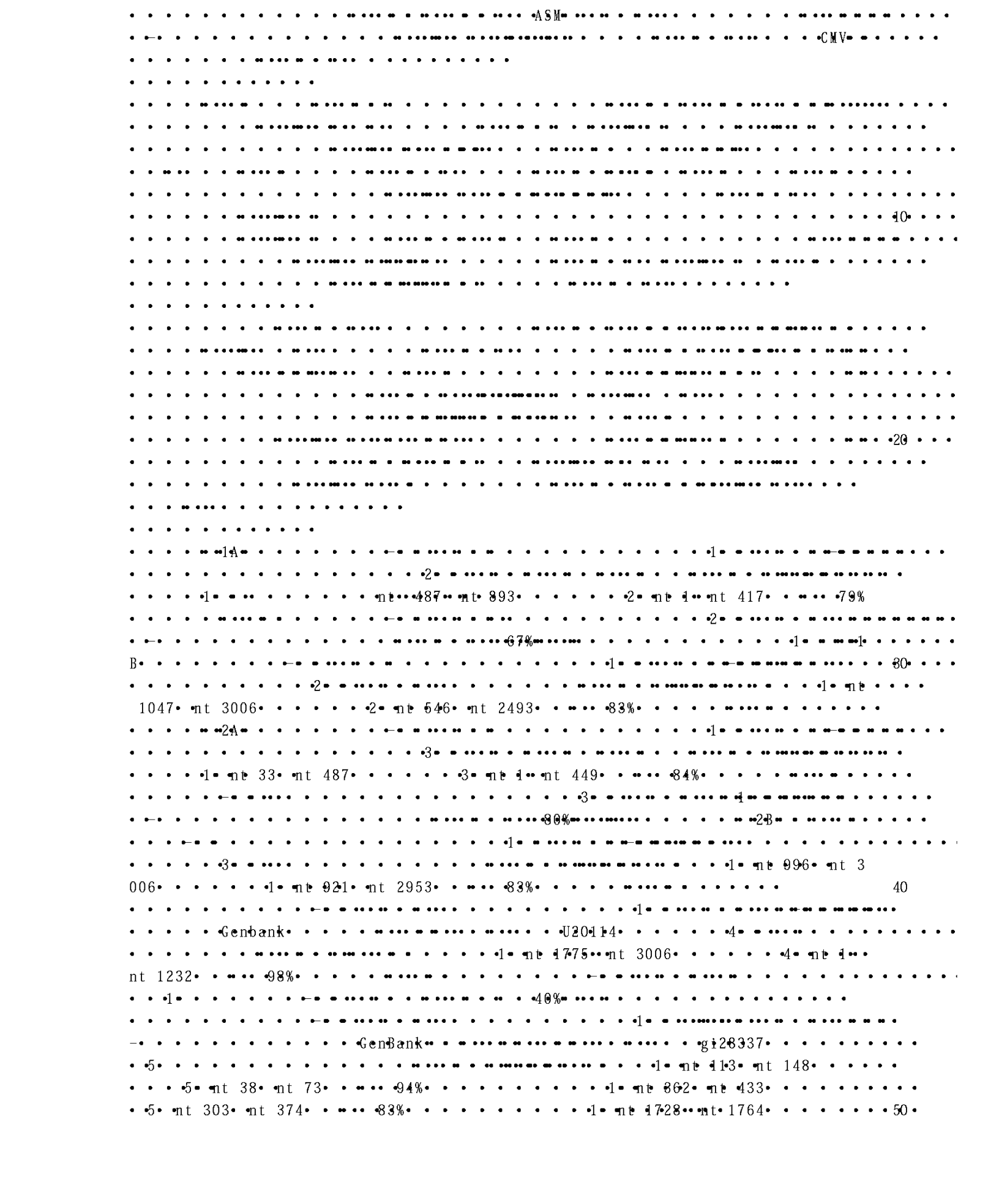
プール	20 nM MTXでのASMの発現	200 nM MTXでのASMの発現
CMV-ASMプールA	4.3	8.2
CMV-ASMプールB	16.9	9.5
CMV-ASMプールC	3.6	3.7
β-アクチン-ASMプールA	33.5	100.0
β-アクチン-ASMプールB	59.3	27.9
β-アクチン-ASMプールC	45.6	90.5

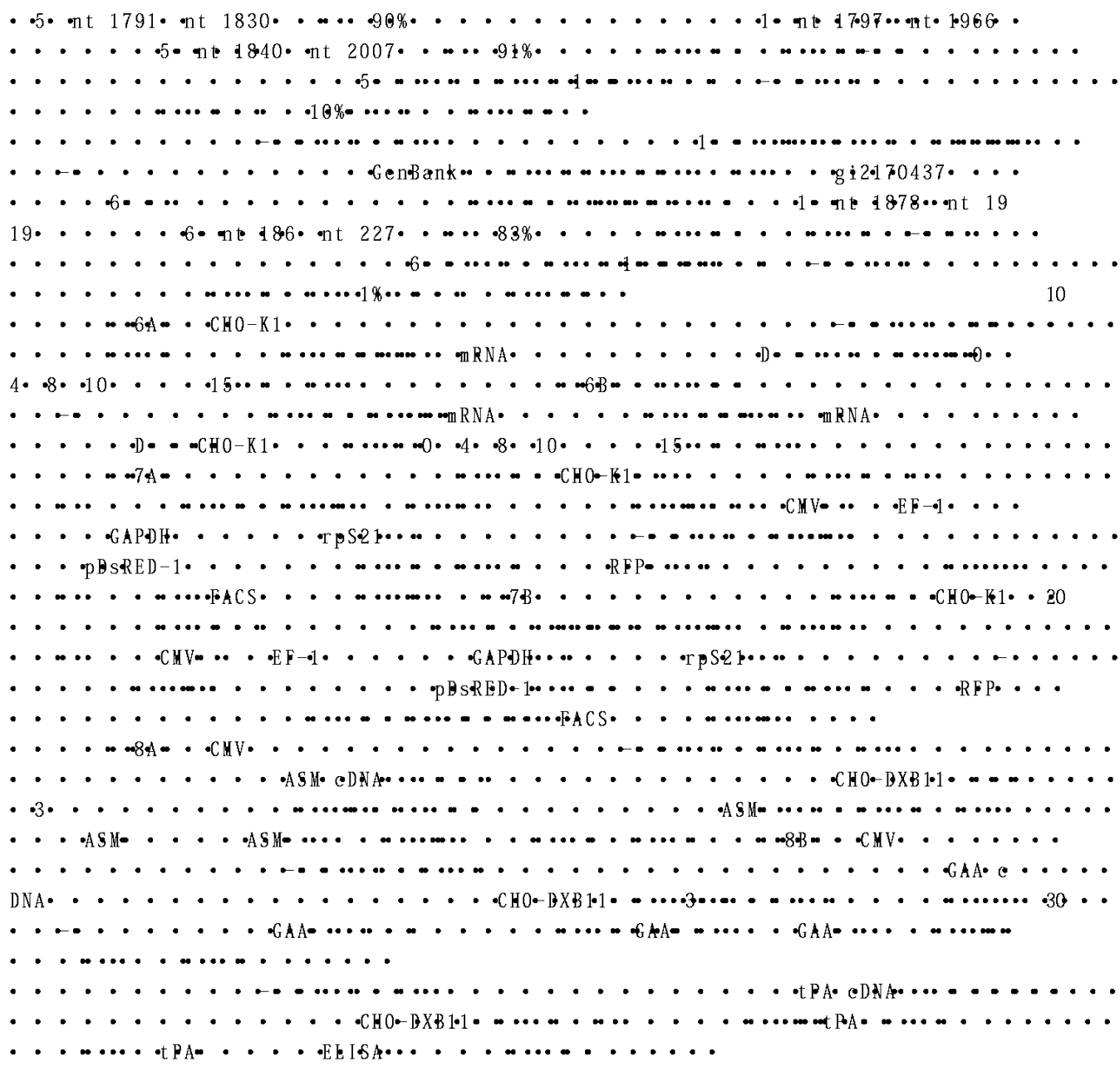
10

ベクター	GAA 発現 <2 pg/細胞/hr	GAA 発現 2-5 pg/細胞/hr	GAA 発現 5-8 pg/細胞/hr	GAA 発現 8-10 pg/細胞/hr
pGZ3IC-GAA	16%	50%	26%	8%
pGZ6IC-GAA	52%	34%	14%	0%

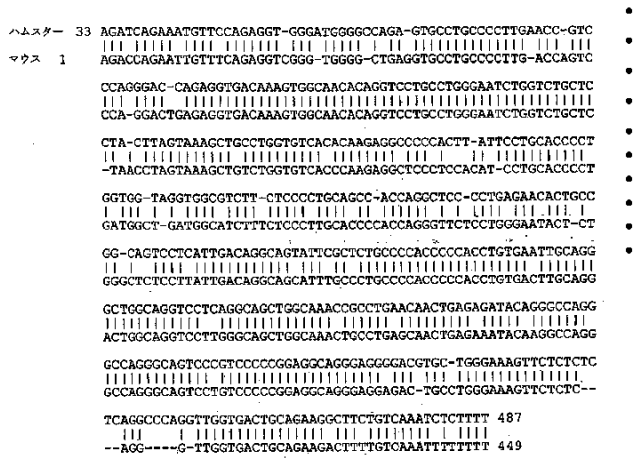
プール	ASM nU/細胞/24 hr (20 nM MTX) の発現	ASM nU/細胞/24 hr (200nM MTX) の発現
rpS21-ASMプールA	12	34
rpS21-ASMプールB	13	30
rpS21-ASMプールC	16	41

プール	ASM発現
CMV-ASMプールA	38
CMV-ASMプールB	193
CMV-ASMプールC	44
β-アクチン-ASMプールA	381
β-アクチン-ASMプールB	125
β-アクチン-ASMプールC	515
rpS21-ASMプールA	342
rpS21-ASMプールB	60
rpS21-ASMプールC	51





ヘルスター 487
ラット1
ヘルスター 1047
ラット546


ハムスター 33 AGATCAGAAATGTTCCAGAGGT-GGGATGGGGCCAGA-GTGCCTGCCCCTTGAACC-GTC
マウス 1 AGACCAGAATTGTTTCAGAGGTCGGG-TGGGG-CTGAGGTGCCTGCCCCTTG-ACCAGTC
CCAGGGAC-CAGAGGTGACAAAGTGGCAACACAGGTCCTGCCTGGGAATCTGGTCTGCTC
CCA-GGACTGAGAGGTGACAAAGTGGCAACACAGGTCCTGCCTGGGAATCTGGTCTGCTC
CTA-CTTAGTAAAGCTGCCTGGTGTCACACAAGAGGCCCCCACTT-ATTCCTGCACCCCT
-TAACCTAGTAAAGCTGTCTGGTGTCACCCAAGAGGCTCCCTCCACAT-CCTGCACCCCT
GGTGG-TAGGTGGCGTCTT-CTCCCCTGCAGCC-ACCAGGCTCC-CCTGAGAACACTGCC
GATGGCT-GATGGCATCTTTCTCCCTTGCACCCCACCAGGGTTCTCCTGGGAATACT-CT
GG-CAGTCCTCATTGACAGGCAGTATTCGCTCTGCCCCACCCCCACCTGTGAATTGCAGG
GGGCTCTCCTTATTGACAGGCAGCATTTGCCCTGCCCCACCCCCACCTGTGACTTGCAGG
GCTGCCAGGTCCTCAGGCAGCTGGCAAACCGCCTGAACAACTGAGAGATACAGGCCCAGG
ACTGGCAGGTCCTTGGGCAGCTGGCAAACTGCCTGAGCAACTGAGAAATACAAGGCCAGG
GCCAGGGCAGTCCCGTCCCCCGGAGGCAGGGAGGGGACGTGC-TGGGAAAGTTCTCTCTC
GCCAGGGCAGTCCTGTCCCCCGGAGGCAGGGAGGAGAC-TGCCTGGGAAAGTTCTCTC--
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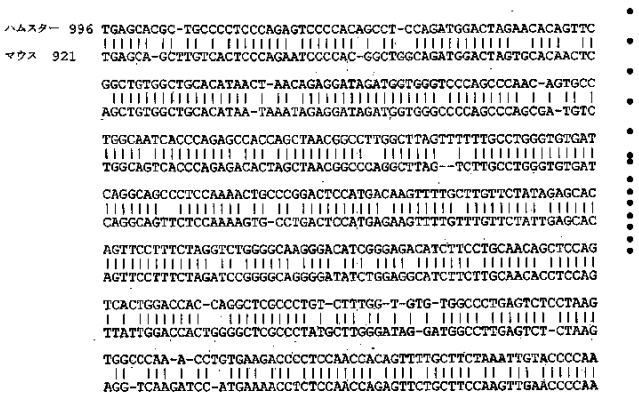
--AGG----G-TTGGTGACTGCAGAAGACTTTTGTCAAATTTTTTTT 449


ハムスター 996 TGAGCACGC-TGCCCCTCCCAGAGTCCCCACAGCCT-CCAGATGGACTAGAACACAGTTC
マウス 921 TGAGCA-GCTTGTCACTCCCAGAATCCCCAC-GGCTGGCAGATGGACTAGTGCACAACTC
GGCTGTGGCTGCACATAACT-AACAGAGGATAGATGGTGGGTCCCAGCCCAAC-AGTGCC
AGCTGTGGCTGCACATAA-TAAATAGAGGATAGATGGTGGGCCCCAGCCCAGCGA-TGTC
TGGCAATCACCCAGAGCCACCAGCTAACGGCCTTGGCTTAGTTTTTTGCCTGGGTGTGAT
TGGCAGTCACCCAGAGACACTAGCTAACGGCCCAGGCTTAG--TCTTGCCTGGGTGTGAT
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CAGGCAGTTCTCCAAAAGTG-CCTGACTCCATGAGAAGTTTTGTTTGTTCTATTGAGCAC
AGTTCCTTTCTAGGTCTGGGGCAAGGGACATCGGGAGACATCTTCCTGCAACAGCTCCAG
AGTTCCTTTCTAGATCCGGGGCAGGGGATATCTGGAGGCATCTTCTTGCAACACCTCCAG
TCACTGGACCAC-CAGGCTCGCCCTGT-CTTTGG-T-GTG-TGGCCCTGAGTCTCCTAAG
TTATTGGACCACTGGGGCTCGCCCTATGCTTGGGATAG-GATGGCCTTGAGTCT-CTAAG
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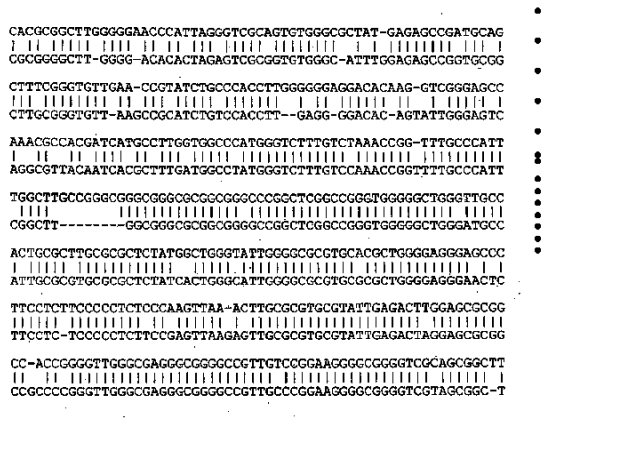
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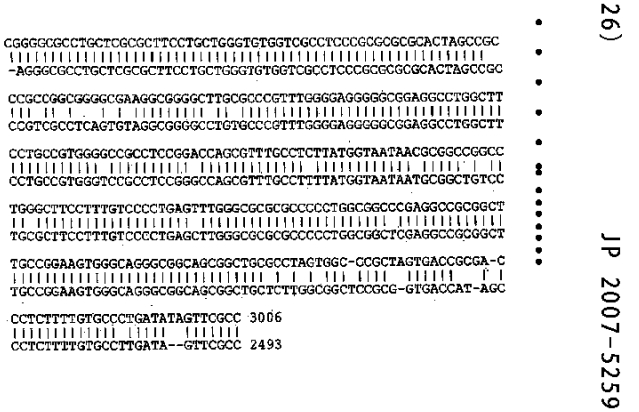
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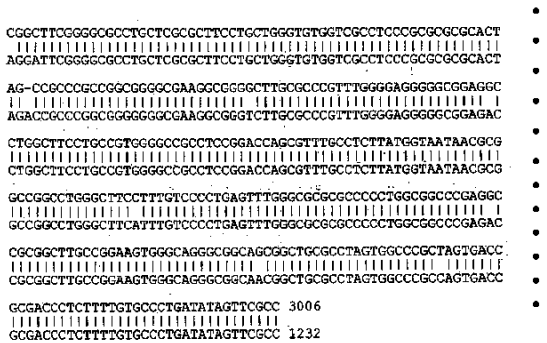
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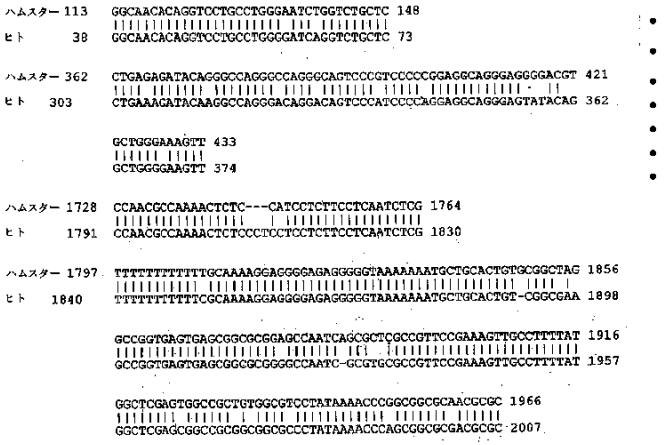
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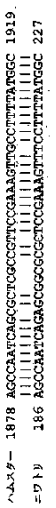
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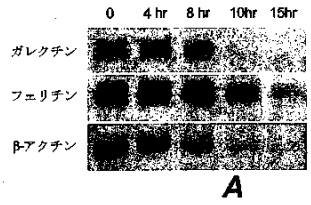
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ガレクチン
フェリチン
β-アクチン

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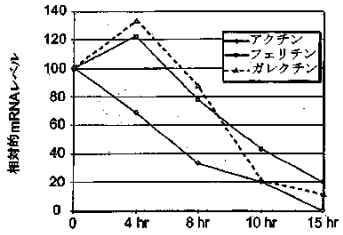
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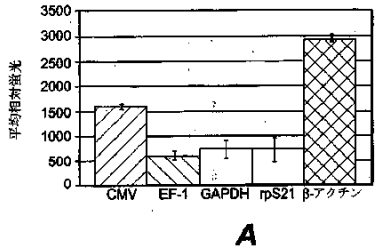
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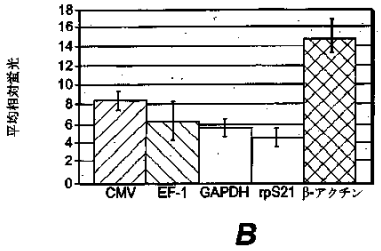
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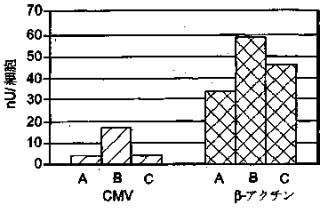
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CMV
A B C
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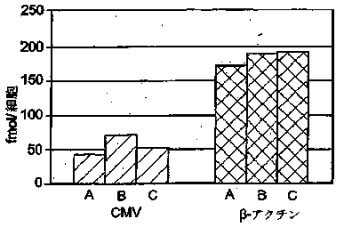
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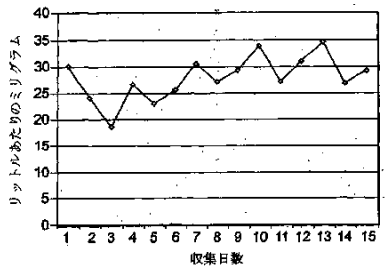

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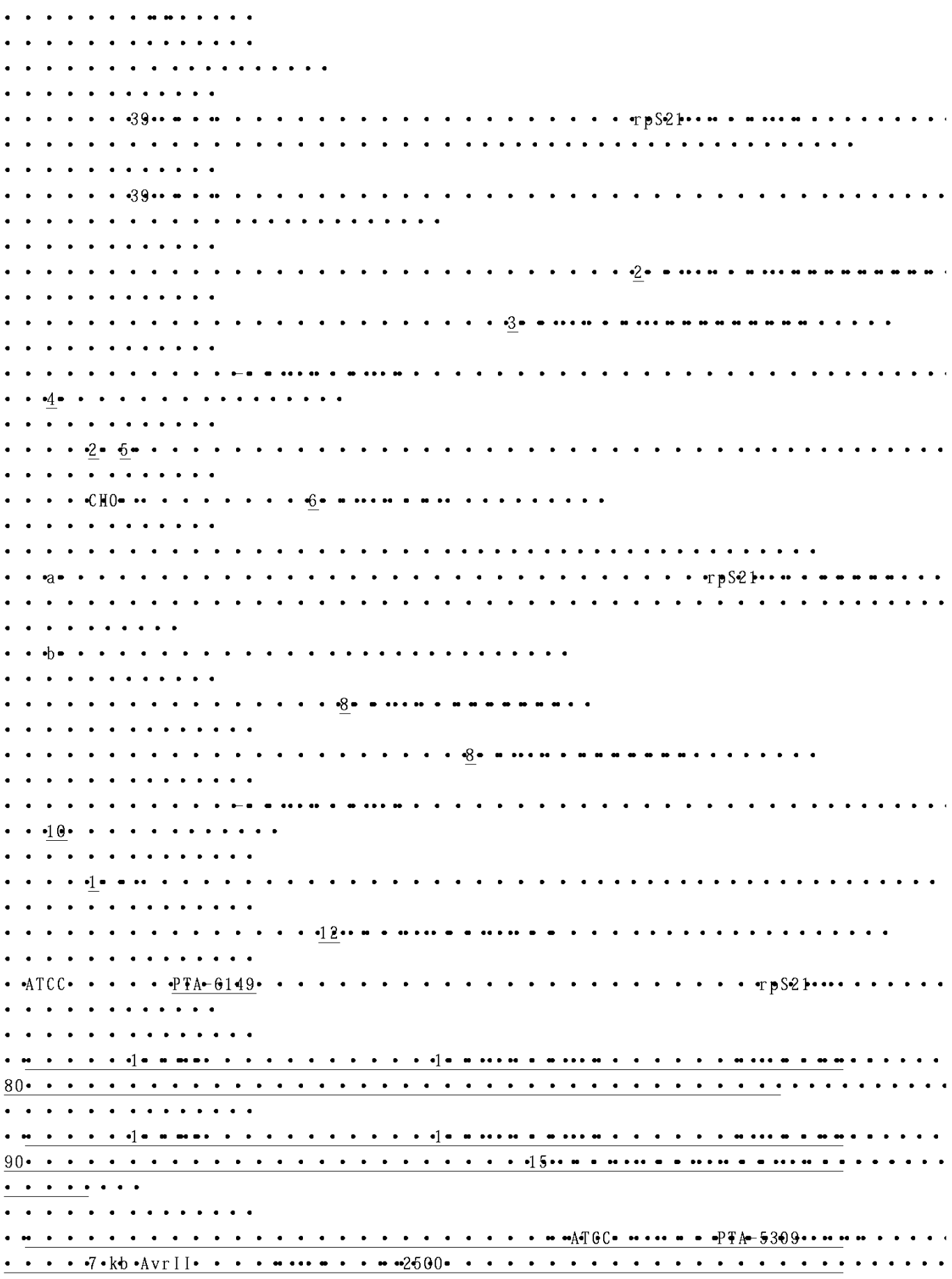
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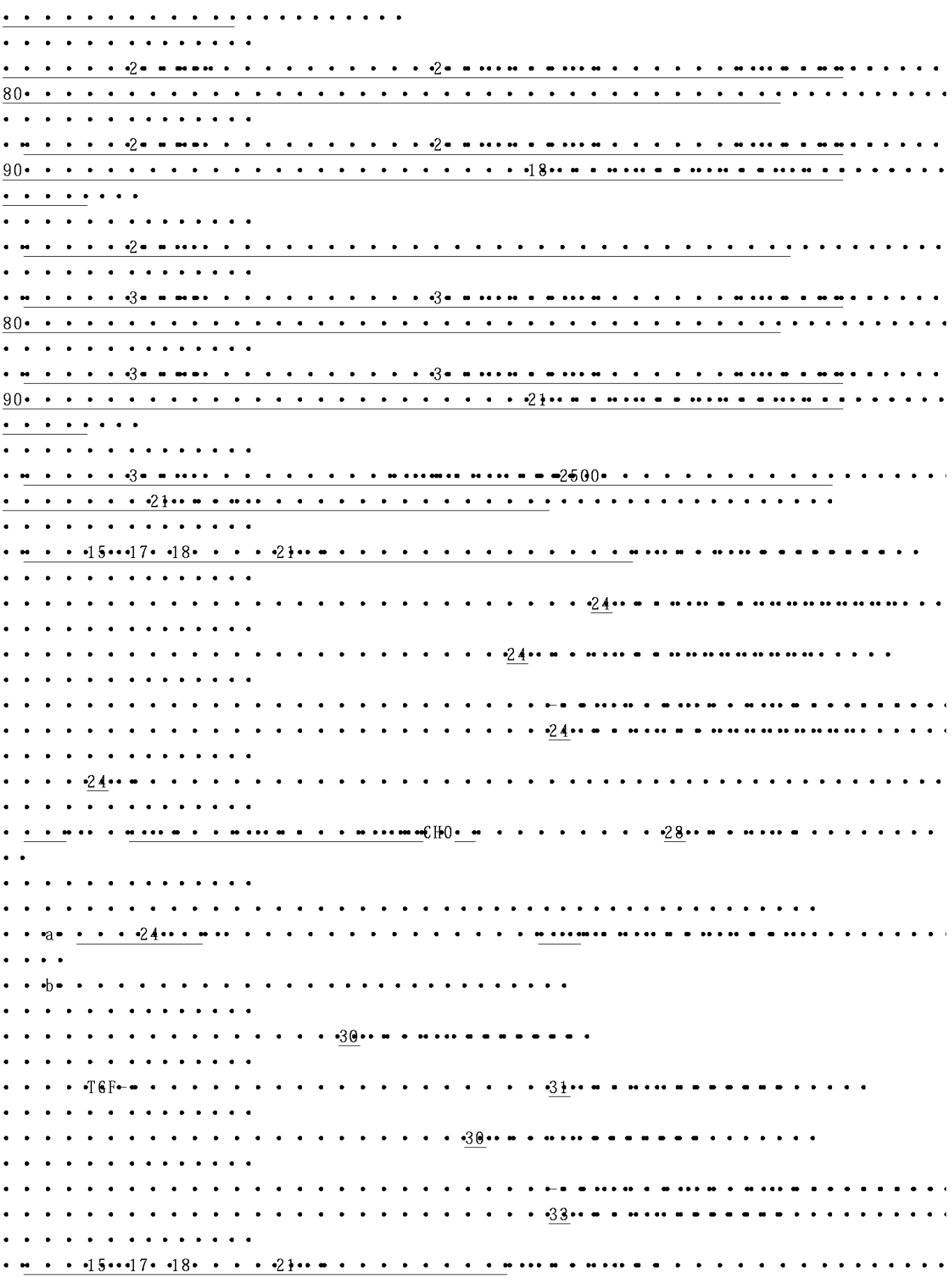

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INTERNATIONAL SEARCH REPORT

Inter application No
PCT/US2004/017422

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/79 C12N15/85 C12N5/10 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEDDINGTON R S P ET AL: "AN IN SITU TRANSGENIC ENZYME MARKER FOR THE MIDGESTATION MOUSE EMBRYO AND VISUALIZATION OF INNER CELL MASS CLONES DURING EARLY ORGANOGENESIS" DEVELOPMENT, COMPANY OF BIOLOGISTS, CAMBRIDGE,, GB, vol. 106, no. 1, 1989, pages 37-46,2, XP001088884 ISSN: 0950-1991	1-10,13, 20,21
Y	page 38, column 1 - column 2; figure 1	1-21,35
	-/—	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents:

'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 December 2004	29.03.05

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mabit, H

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Int nal application No.
PCT/US2004/017422

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-21, 35

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International Application No. PCT/US2004 /017422

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-21, 35

an isolated rodent beta actin promoter chosen from nucleotide sequences set forth in SEQ ID N°1, 2, and 3, or a varinat thereof having promoter activity, and subject-matter related thereon

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2. claims: 22-34, 36

an isolated rpS21 promoter having the nucleotide sequence set forth in SEQ ID N°39 and subject-matter related thereon

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INTERNATIONAL SEARCH REPORT

Inte plication No
PCT/US2004/017422

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BREITBART A S ET AL: "Gene-enhanced tissue engineering: applications for wound healing using cultured dermal fibroblasts transduced retrovirally with the PDGF-B gene." ANNALS OF PLASTIC SURGERY. DEC 1999, vol. 43, no. 6, December 1999 (1999-12), pages 632-639, XP009042066 ISSN: 0148-7043	1-11,13
Y	page 635, column 2, paragraph 3; figure 1	1-21,35
Y	NUDEL U ET AL: "The nucleotide sequence of the rat cytoplasmic beta-actin gene." NUCLEIC ACIDS RESEARCH. 25 MAR 1983, vol. 11, no. 6, 25 March 1983 (1983-03-25), pages 1759-1771, XP002312115 ISSN: 0305-1048 page 1764- - page 1765; figure 2	1-21,35
Y	-& DATABASE EMBL EBI; 13 July 1983 (1983-07-13), NUDEL U ET AL.,: "The nucleotide sequence of the rat cytoplasmic beta-actin gene" XP002312127 retrieved from EBI Database accession no. V01217 the whole document	1-21,35
Y	DATABASE EMBL EBI; 21 April 1995 (1995-04-21), STAHLBOM PA AND FRANZEN SA: "Isolation and characterization of the beta actin gene from chinese hamster" XP002312117 retrieved from EBI Database accession no. U20114 the whole document	1-21,35
Y	ELDER P K ET AL: "EVIDENCE THAT THE FUNCTIONAL BETA ACTIN GENE IS SINGLE COPY IN MOST MICE AND IS ASSOCIATED WITH 5' SEQUENCES CAPABLE OF CONFERRING SERUM AND CYCLOHEXIMIDE-DEPENDENT REGULATION" MOLECULAR AND CELLULAR BIOLOGY, vol. 8, no. 1, 1988, pages 480-485, XP002312116 ISSN: 0270-7306 page 480, column 2 - page 481, column 1	1-21,35

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Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Interr plication No
PCT/US2004/017422

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIM TEOAN ET AL: "Gene transfer in bovine blastocysts using replication-defective retroviral vectors packaged with gibbon ape leukemia virus envelopes" MOLECULAR REPRODUCTION AND DEVELOPMENT, vol. 35, no. 2, 1993, pages 105-113, XP009042067 ISSN: 1040-452X page 106, column 2, last paragraph - page 108, column 2, paragraph 2; table 2	1-21,35
A	NAKAJIMA-IIJIMA S ET AL: "MOLECULAR STRUCTURE OF THE HUMAN CYTOPLASMIC BETA ACTIN GENE INTERSPECIES HOMOLOGY OF SEQUENCES IN THE INTRONS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 82, no. 18, 1985, pages 6133-6137, XP002312177 ISSN: 0027-8424	

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

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(51)Int.Cl. • • • • • • • • • • • • • • • • • • •

• • • • • 21/08 (2006.01) • • • • • 21/08 • • • • • • • • • • • • • • • • • •

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